RHODORA, Vol. 95, No. 883/884, pp. 254–260, 1993

INTRASPECIFIC TAXONOMY AND COMPARISONS OF NRDNA ITS-2 SEQUENCES OF *ARISAEMA RINGENS* (ARACEAE)

SUNG CHUL KO,[1] STEVE L. O'KANE, JR., AND BARBARA A. SCHAAL

ABSTRACT

The second internal transcribed spacer (ITS-2) of ribosomal DNA (rDNA) was sequenced for the three phenotypes of *Arisaema ringens* (Araceae). These sequences were invariant, and are therefore not systematically informative. However, when considered along with the anatomical, morphological, cytological, and palynological data summarized here, the recognition of intraspecific taxa in *A. ringens* is not supported.

Key Words: *Arisaema,* nrDNA, ribosomal DNA, transcribed spacer, ITS-2

INTRODUCTION

Arisaema ringens (Thunberg) Schott (section Pistillata; Araceae), Japan, China and S. Korea, was divided by Engler (1879) into two varieties, *α sieboldii* and *β praecox,* based on cataphyll and petiole color. The former has green cataphylls and pale violet petioles, and the latter has red cataphylls and pale green petioles. Workers have had varying opinions of these taxa. Palibin (1901) and Chung (1957) agreed with Engler in maintaining two varieties. Other taxonomists have viewed these phenotypes as different species (e.g., De Vriese, 1839; Nakai, 1952) or, more recently, united them into a single species (e.g., Ohwi and Kitagawa, 1983; Ohashi and Murata, 1980; Wu and Li, 1979). Koyama (1984) made Engler's varieties into forms based on spathe color rather than cataphyll and petiole color, i.e., *A. ringens* f. *sieboldii* with dark purple spathes and *A. ringens* f. *praecox* with greenish spathes. Ko and Kim (1985) agreed with Koyama.

In the field three phenotypes of *Arisaema ringens* are found. Based on the current intraspecific taxonomy, these phenotypes may be separated as follows: individuals with purple spathe margins and petioles [var. *sieboldii* (de Vriese) Engl.], individuals with purple spathe margins and green petioles [var. *praecox* (de Vriese)

[1] Current address: Dept. of Biology, Han-nam University, 133 O-jung dong Tae-jun, Korea.

Engl.], and individuals with greenish spathe margins and green petioles (f. *praecox* T. Koyama). If, however, petiole color is recognized as a good taxonomic character, then characters of var. *sieboldii* and var. *praecox* are found within f. *sieboldii*.

Several recent studies have examined the intraspecific systematics of *Arisaema ringens*. Morphology and geographical distribution are treated in Ko and Kim (1985). Cytology, anatomy and palynology have also been studied (Ko et al., 1990; Oh et al., 1990; Ko et al., 1987; Ko and Kim, 1985; Hotta, 1971; and Ito, 1942). In order to further clarify the intraspecific relationships in *A. ringens* we examined the sequence of one of the internal spacers of large subunit rDNA (ITS-2). DNA sequences have proved extremely useful in other taxonomic studies (Crawford, 1990). The coding regions of rDNA (18S, 5.8S, and 28S) are not useful at the intraspecific level because they evolve very slowly in sequence and almost not at all in length. These sequences are particularly useful at the level of family and above (Baverstock and Johnson, 1990; Schaal and Learn, 1988). The intergenic spacer (IGS) evolves rapidly both in sequence and length, making it difficult to use except at the population level (Learn and Schaal, 1987; Jorgensen and Cluster, 1988). The ITS regions (ITS-1 and ITS-2), however, have an intermediate level of variation (Baldwin, 1992a; Schaal and Learn, 1988; Hamby and Zimmer, 1992) that makes them ideal for use at or below the generic level. No studies have reported ITS sequences at the subspecific level; at the specific level, percent sequence divergence ranges from 0.4% to 12.9%, excluding the single value of 0% for variation within *Madia bolanderi* (Baldwin, 1992a).

METHODS

Plants were grown in the Washington University greenhouse from corms collected in the Jeon-La-Nam-Do Province of Korea. Plants with purple spathes and purple petioles and peduncles were from near Dae-Heung Temple, Hae-Nam Kum. Plants with green or purple spathes and green petioles and peduncles were from Kun-Oe Myun, Wan-Do (Island) Kun. DNA was extracted from fresh tissue using the CTAB procedure of Doyle and Doyle (1987) with the addition of a phenol extraction. Primers "ITS-3" (GCATCGATGAAGAACGTAGC) and "ITS-4" (TCCTCC-GCTTATTGATATGC) for PCR amplification of ITS-2 were

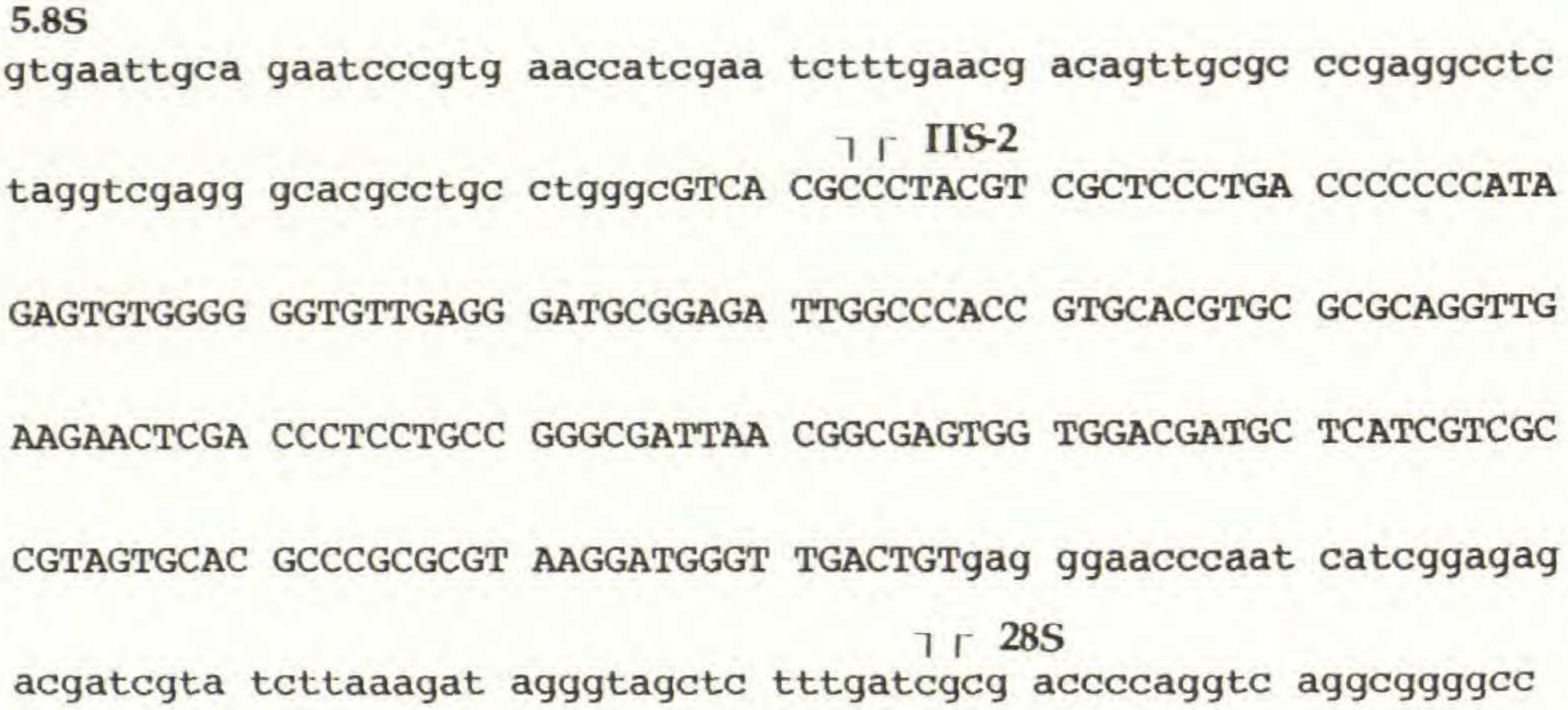

Figure 1. Nucleotide sequence of the rDNA ITS-2 region of the three phenotypes of *Arisaema ringens* including the 28S rDNA flanking region and most of the 5.8S rDNA. Overlap sequence from primers 3 and 4 is indicated in capitals.

constructed from the consensus sequences reported by White et al. (1990). The fourth base (C) from the 5′ end of primer "ITS-3" was changed to T to match the sequences for flowering plants reported elsewhere (e.g., Yokata et al., 1989). Both strands of the double stranded PCR products were directly sequenced using Sequenase (US Biochemicals). Sequence overlap was obtained for 52.5 percent of the total sequence and 75.4 percent of ITS-2 (Figure 1). Sequencing protocols are essentially those given by US Biochemicals except that labeling and extension reactions were performed in a single step, Sequenase was diluted 1:6 rather than 1:8, and termination reactions were carried out at 49°C. The entire sequence of ITS-2 and most of the 5.8S rDNA was sequenced for one individual of each phenotype.

RESULTS

The sequences obtained for the three phenotypes were identical, indicating to us that no further sequences need be sampled for *Arisaema ringens*, especially as the three samples are from two different locations. ITS-2 was found to be 244 bases long with a G+C content of 61.5 percent (Figure 1). These values are similar to other reported ITS-2 sequences (Yokata et al., 1989; Kavanagh and Timmis, 1988; Takaiwa et al., 1985). The location of the ends of the 5.8S and 26S sequences are based on comparisons

Table 1. Summary of characters of *Arisaema ringens* var. *praecox,* var. *sieboldii,* and f. *praecox.*

	Taxon		
	var. *praecox*	var. *sieboldii*	f. *praecox*
Morphology			
Peduncle	green	purple	green
Petiole	green	purple	green
Spathe	purple	purple	green
Cytology[1]			
Chromosome no. ($2n$)	28	28	—
Karyotype			
Relative size			
Very long	1	1	—
Long	9	8	—
Medium	1	2	—
Small	3	3	—
Total chromosomal length (μm)			
Ave.	6.57	3.31	—
Range	3.95–8.83	2.07–4.35	—
Type			
Metacentric	2	3	—
Submetacentric	12	10	—
Subtelocentric	0	1	—
Anatomy—Cell wall specialization index[2]			
Peduncle	2.10	1.50	
Petiole	3.75	2.90	—
Root	3.90	4.85	—
Ave.	3.25	3.08	—

[1] Ko, S. C. & Y. S. Kim, 1985.
[2] Ko, S. C. et al., 1990.

with published ITS-2 sequences (Rathgeber and Capesius, 1989; Yokata et al., 1989; Kavanagh and Timmis, 1988; Kiss et al., 1988; Takaiwa et al., 1985).

DISCUSSION AND CONCLUSIONS

The intraspecific taxonomy of *Arisaema,* as noted previously, is currently confused, especially so if we wish to recognize the three variable but non-overlapping phenotypes recognizable in the field. In addition the names of these phenotypes are non-heirarchical, i.e., var. *praecox,* var. *sieboldii,* and f. *praecox.* Table 1 summarizes the anatomy, cytology, and morphology of the

intraspecific taxa of *Arisaema ringens*. *Forma praecox* has not been the subject of previous studies other than morphology.

Cytologically, *Arisaema ringens* var. *praecox* and var. *sieboldii* are very similar, both having $2n = 28$ chromosomes and nearly identical karyotypes (Table 1). Although they share a pair of chromosomes that is slightly different from one another in length and chromosome type, these differences are somewhat subjective, e.g., chromosome length is dependent on tissue condition and age of tissue as well as parameters of staining. Geographically these taxa occur in the same subtropical to warm temperate region. Although electron density of the pollen ektexine of var. *sieboldii* is somewhat greater than in var. *praecox*, there are no significant differences in pollen size, shape, and wall architecture. Cross-sections of roots, petioles, petiolules, peduncles, and leaves show the same tissue arrangement and level of tissue specialization and differentiation in both taxa. Diameter and cell wall thickness of tracheids, and diameter and length of fibers in both taxa are not significantly different, and these characters have been shown not be phylogenetically useful in *Arisaema* (Ko et al., 1990). Based on the level of specialization of cell wall thickenings, var. *sieboldii* is slightly more advanced in its root anatomy and var. *praecox* is slightly more advanced in areal organs. Although the distribution of colors of peduncle, petiole, and spathe are not overlapping, they are quite variable within a given taxon.

Many morphological characters of plants, some of them quite striking, have been shown to be under the control of alleles of one or a few genes (Coen and Meyerowitz, 1991; Gottlieb, 1984; Hilu, 1983). It is probable that colors thought to distinguish intraspecific taxa of *Arisaema ringens* are similarly controlled and represent intraspecific polymorphism rather than synapomorphies of evolutionary lineages. Sequences of ITS-2 show no differences among the intraspecific taxa of *Arisaema ringens*. These sequences are known to be good markers of evolutionary lineages at or near the species level (Baldwin, 1992b; Baldwin et al., 1992; Suh et al., 1992). Insignificant difference in morphology and anatomy, and the lack of ITS-2 sequence variation, suggests that the intraspecific taxa of *Arisaema ringens* should be consolidated and a single polymorphic taxon should be recognized. Further work is required to assay levels of ITS-2, as well as ITS-1, variation within and among other species of *Arisaema*.

LITERATURE CITED

BALDWIN, B. G. 1992a. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. Mol. Phylog. Evol. 1: 3–16.

———. 1992b. Phylogenetics of *Calycadenia* (Compositae-Madiinae) based on nuclear 18-26S rDNA intercistronic transcribed spacer (ITS) sequences. Amer. J. Bot. 79(Supp.): 134(abstract).

———, R. H. ROBICHAUX, G. D. CARR AND D. W. KYHOS. 1992. Evolutionary and biogeographic patterns in the Hawaiian silversword alliance (Compositae-Madiinae). Amer. J. Bot. 79(Supp.): 125(abstract).

BAVERSTOCK, P. R. AND A. M. JOHNSON. 1990. Ribosomal RNA nucleotide sequence: a comparison of new methods used for its determination, and its use in phylogenetic analysis. Austral. Syst. Bot. 3: 101–110.

COEN, E. S. AND E. M. MEYEROWITZ. 1991. The war of the whorls: genetic interactions controlling flower development. Nature 353: 31–37.

CHUNG, T. H. 1957. Korean Flora II. Shinzisa, Seoul. Pp. 776–779.

CRAWFORD, D. J. 1990. Plant Molecular Systematics. Wiley and Sons, New York.

DE VRIESE, W. H. 1839. Hortus spaaren-bergensis. I. *In:* A. Engler DC. Monographiae Phanerogamarum 2: 533–560.

DOYLE, J. J. AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

ENGLER, A. 1879. *Arisaema. In:* DC., Monographiae Phanerogarum. Masson, Paris. 2: 533–560.

GOTTLIEB, L. D. 1984. Genetics and mophological evolution in plants. Amer. Naturalist 123: 681–709.

HAMBY, R. K. AND E. A. ZIMMER. 1992. Ribosomal RNA as a phylogenetic tool in plant systematics. *In:* P. S. Soltis, D. E. Soltis and J. J. Doyle, Eds., Molecular Systematics of Plants. Chapman and Hall, New York. Pp. 50–91.

HILU, K. W. 1983. The role of single-gene mutations in the evolution of flowering plants. Evol. Biol. 16: 97–128.

HOTTA, M. 1971. Study of the family Araceae, general remarks. Jap. J. Bot. 20: 269–310.

ITO, T. 1942. Chromosomen und Sexualität vonder Araceae, I. Somatische Chromosomen-Zahlen einiger Arten. Cytologia 12: 313–325.

JORGENSEN, R. A. AND P. D. CLUSTER. 1988. Modes and tempos in the evolution of nuclear ribosomal DNA: new characters for evolutionary studies and new markers for genetic population studies. Ann. Missouri Bot. Gard. 75: 1238–1247.

KAVANAGH, T. A. AND J. N. TIMMIS. 1988. Structure of melon rDNA and nucleotide sequence of the 17-25S spacer region. Theor. Appl. Genet. 76: 673–680.

KISS, T., M. KIS, S. ABEL AND F. SOLYMOSY. 1988. Nucleotide sequence of the 17S-25S spacer region from tomato rDNA. Nuc. Acids Res. 16: 7179.

KO, S. C. AND Y. S. KIM. 1985. A taxonomic study of genus *Arisaema* in Korea. Korean J. Plant. Tax. 15: 67–109.

———, B. U. OH, H. S. LEE AND Y. S. KIM. 1990. A phylogenetic study of

Arisaema by anatomical and palynological characters. I. Anatomical characters. Korean J. Plant Tax. 20: 9–35.

———, K. H. Tae, T. O. Kwon and Y. S. Kim. 1987. A cytotaxonomic study on some species of *Arisaema* Korean J. Plant Tax. 17: 189–205.

Koyama, T. 1984. *Arisaema,* pp. 256–262. *In:* F. G. Meyer and E. H. Walker, Eds., J. Ohwi, Flora of Japan. Smithsonian Inst. Washington, D.C.

Learn, G. H. and B. A. Schaal. 1987. Population subdivision for ribosomal DNA repeat variants in *Clematis fremontii.* Evol. 41: 433–438.

Nakai, T. 1952. A synoptical sketch of Korean flora. Bull. Nat. Sci. Mus. 31: 1–152.

Oh, B. U., S. C. Ko, W. P. Hong and Y. S. Kim. 1990. A phylogenetic consideration of *Arisaema* by anatomical and palynological characters. II. Palynological characters. Korean J. Plant Tax. 20: 37–42.

Ohashi, H. and J. Murata. 1980. Taxonomy of the Japanese *Arisaema.* J. Fac. Sci. Univ. Tokyo, Sect. S, Bot. 12: 281–336.

Ohwi, J and M. Kitagawa. 1983. New Flora of Japan. Shibundo, Tokyo. Pp. 350–351.

Palibin, J. 1901. Conspectus Flora Korea III. Act. Hort. Petrop. 19: 101–151.

Rathgeber, J. and I. Capesius. 1989. Nucleotide sequence of the 18S-25S spacer region from mustard DNA. Nucl. Acids Res. 17: 7522.

Schaal, B. A. and G. H. Learn. 1988. Ribosomal DNA variation within and among plant populations. Ann. Missouri Bot. Gard. 75: 1207–1216.

Suh, Y, L. B. Thein and E. A. Zimmer. 1992. Molecular evolution of the internal transcribed spacers of nuclear ribosomal DNA and a phylogeny of the Winteraceae. Amer. J. Bot. 79(Supp.): 163(abstract).

Takaiwa, F. O. Kiyoharu and M. Sugiura. 1985. Nucleotide sequence of the 17S-25S spacer region from rice rDNA. Pl. Mol. Biol. 4: 355–364.

White, T. J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, pp. 315–322. *In:* M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White, Eds., PCR Protocols. Academic Press, New York.

Wu, C. Y. and H. Li. 1979. Flora Reipublicae Popularis Sinicae. Institutum Botanicum Kummingense Academiae Sinicae. Tormus 13: 116–194.

Yokata, Y., T. Kawata, Y. Iida, A. Kato and S. Tanifuji. 1989. Nucleotide sequences of the 5.8S rRNA gene and internal transcribed spacer regions in carrot and broad bean ribosomal DNA. J. Mol. Evol. 29: 294–301.

DEPARTMENT OF BIOLOGY, CAMPUS BOX 1137
WASHINGTON UNIVERSITY
ST. LOUIS, MO 63130